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9.0 Animal Welfare Considerations (Refinement, Reduction, and Replacement)

9.1 Refinement, Reduction, and Replacement Considerations

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible. Refinement, Reduction, and Replacement are known as the three "Rs" of animal alternatives. These principles of humane treatment of laboratory animals are described as:

- Refining experimental procedures such that animal suffering is minimized
- Reducing animal use through improved science and experimental design
- Replacing animal models with non-animal procedures (e.g., *in vitro* technologies), where possible (Russell and Burch 1959)

There are currently three *in vivo* methods commonly used by regulators to assess the estrogenic potential of substances: rat uterotrophic, rat pubertal female, and fish short-term reproduction assay. In addition, the "*in vitro*" Rat Uterine Cytosol ER binding assay also requires the use of animals as a source of ER. Although the BG1Luc ER TA will not directly replace any of these existing methods, it could be incorporated as part of a weight of evidence approach to reduce or eliminate the need for testing in these animal models. There currently are no accepted validated *in vitro* test methods in use for the screening of both ER agonists and antagonists (ICCVAM 2002). As stated in **Section 1.0**, the EPA EDSP Tier 1 screening battery currently includes the CERI STTA agonist test method, *OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903))*. The screening guideline also makes provisions for the use of other scientifically valid methods. Therefore, the BG1Luc ER TA may be applicable for addressing the ER TA component of the EPA EDSP Tier 1 screening battery. Used in this context, the BG1Luc ER TA provides an opportunity to reduce animal use in ED testing by identifying substances that may enhance and/or inhibit the activation of the ER.

The BG1Luc4E2 ER TA method is being proposed as an independent part of a weight-of-evidence approach to prioritize potentially endocrine active substances for further testing. Therefore, like the CERI STTA, the test does not directly refine or replace animal use. However, there are currently three *in vivo* methods commonly used by regulators to assess the estrogenic potential of substances: rat uterotrophic, rat pubertal female, and fish short-term reproduction assay. In addition, the "*in vitro*" Rat Uterine Cytosol ER binding assay also requires the use of animals as a source of ER. Results from the BG1Luc ER TA were examined for concordance with published reports of ER binding. There was 97% (33/34) concordance between the BG1Luc ER TA and ER binding data from the literature (see **Section 5.6**). In

light of the excellent degree of agreement between ER binding and BG1Luc ER TA (with no false negative results), it appears that evaluating results from BG1Luc ER TA agonist and antagonist testing may provide a viable alternative to conducting ER binding studies, which use animals as a source of ER . This cannot currently be accomplished with the only accepted ER TA method due to the inability of the CERI STTA method to assess ER antagonist activity.

Results from the BG1Luc ER TA were examined for concordance with published data from the uterotrophic assay (see **Section 5.7**). Based on a comparison with the *in vivo* uterotrophic assay classification, the 13 substances with data from the uterotrophic assay and conclusive test results in the BG1Luc ER TA agonist test method produced overall concordance of 92% (12/13). All substances found positive in the uterotrophic assay were also positive in the BG1Luc ER TA method. The only discordant substance, butylbenzyl phthalate was positive for ER agonist activity in the BG1Luc ER TA agonist test method and negative in the uterotrophic assay. These data indicate that the BG1Luc ER TA agonist test method has very good agreement with the *in vivo* results obtained with the uterotrophic assay, with no false negative results.

Although the BG1Luc ER TA will not directly replace any of these existing methods, it could be incorporated as part of a weight of evidence approach to reduce or eliminate the need for testing in these animal models.

9.2 Use of Animals in the BG1Luc ER TA

The BG1Luc ER TA test method utilizes cultured human ovary adenocarcinoma cells that endogenously express human ER and contains an estrogen-inducible gene expression system. Except for the fetal bovine sera used as part of the cell culture media, the test method does not require the use of animals.

ICCVAM. 2002. Background Review Document. Current Status of Test Methods for Detecting Endocrine Disruptors: In Vitro Androgen Receptor Transcriptional Activation Assays.National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/arta_brd/ARTA034507.pdf

Russell WMS, Burch RL. 1959. The Principles of Humane Experimental Technique. London:Methuen & Co. Ltd. [Reissued: 1992, Universities Federation for Animal Welfare, Herts England.].